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Characterization of the microbial community in biological soil crusts dominated by *Fulgensia desertorum* (Tomin) Poelt and *Squamarina cartilaginea* (With.) P. James and in the underlying soil

Felipe Bastida [a,*], Nico Jehmlich [b], Sara Ondoño [a], Martin von Bergen [b,c,d], Carlos García [a], José L. Moreno [a]

[a] Department of Soil and Water Conservation, Centro de Edafología y Biología Aplicada del Segura, CEBAS-CSIC, Campus Universitario de Espinardo, Aptdo. de Correos 164, Espinardo, 30100 Murcia, Spain
[b] Department of Proteomics, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, D-04318 Leipzig, Germany
[c] Department of Metabolomics, Helmholtz Centre for Environmental Research – UFZ, Permoserstr. 15, 04318 Leipzig, Germany
[d] Aalborg University, Department of Biotechnology, Chemistry and Environmental Engineering, Sohngårdsholmsvej 49, 9000 Aalborg, Denmark

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ABSTRACT

In arid and semiarid areas of the planet, biological soil crusts (BSCs) participate in functions critical to the ecosystem sustainability and are usually located in inter-vegetation spaces. These crusts are structurally and in terms of biomass dominated by mosses and lichens. Associated with them, a great variety of fungi, bacteria, and cyanobacteria have been found. Despite their ecological importance, major details of the functionalities of the microbial communities inhabiting BSCs and the underlying soil are still missing. Here, we explore the biomass, functionality, and protein-based phylogeny of the microbial communities associated with lichen-dominated BSCs under a semiarid climate. Two lichen-dominated BSCs (*Fulgensia desertorum* -dominated BSC, F-BSC; and *Squamarina cartilaginea* -dominated BSC, S-BSC) were sampled in a semiarid soil located in South-East Spain. Samples of the BSCs and the underlying soils were collected. The biomass and activity of the microbial community in both BSCs was higher than in the soil underlying them, as estimated by their PLFA content, basal respiration, and enzyme activities. The factor analysis of PLFAs and the putative phylogenetic analysis of the identified proteins indicated scarce differences in the composition of the microbial communities of the two biocrusts at the phylum level. In contrast, community-level physiological profiles (CLPPs) revealed that the microbial communities inhabiting BSCs metabolized substrates differently. The identification of ribulose-1,5 biphosphate carboxylase oxygenase (RuBisCO) peptides points to a specialization in carbon fixation which is carried out by some cyanobacterial species in F-BSC, such as *Prochlorothrix hollandica* and *Microcoleus vaginatus*, but not in S-BSC. The structure of the microbial community in the soil underlying each BSC was different, as estimated by factor analysis of PLFAs, but such differences did not correspond to changes in the functional structure, as indicated by the CLPPs. The multidisciplinary approach used in this study revealed remarkable differences between the microbial communities inhabiting *F. desertorum* and *S. cartilaginea* -dominated biological crusts, whereas the microbial communities in the soils underlying the two crusts behaved similarly.

1. Introduction

Extensive areas of soil under arid and semiarid conditions are not covered by vegetation and hence are subjected to intensive processes of desertification (García et al., 1994; Bastida et al., 2006). In such areas, commonly the only macroscopic soil organisms are the biological soil crusts (BSCs), which constitute a dominant biotic component (Belnap and Lange, 2003) composed mainly of mosses, lichens, fungi, and cyanobacteria (Pointing and Belnap, 2012). Biological soil crusts normally constitute a thin mantle of the soil, but their impact on ecosystem functioning can be quite strong (Belnap and Lange, 2003). The BSCs are related to the hydrological cycle (Belnap et al., 2005), improve the physical structure of soil and hence reduce soil erosion (Belnap and Gillete, 1998), and play an

* Corresponding author.
E-mail address: fbastida@cebas.csic.es (F. Bastida).

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important role in the bio-geochemistry of elements (Maestre and Cortina, 2003; Castillo-Monroy et al., 2010; Miralles et al., 2012a). For instance, BSCs are responsible for autotrophic carbon fixation and nitrogen fixation in drylands (Bowker et al., 2011).

Although the organisms constituting BSCs may influence the biomass and structure of the surrounding microbial communities (Yeager et al., 2004; Nagy et al., 2005; Gundlapally and García-Pichel, 2006; Zaady et al., 2010; Castillo-Monroy et al., 2011a; Johnson et al., 2012), the relationships between BSCs and microbial populations remain poorly understood. Bowker et al. (2010) observed that indicators of carbon and phosphorus cycling were apparently suppressed by the lichen *Diploschistes diacapsis* (Ach.) Lumbsch and promoted by *Squamarina lentigera* (G.H. Weber) Poelt, respectively. Moreover, Castillo-Monroy et al. (2011b), using a DGGE approach, concluded that lichen-dominated BSC communities had a strong influence on the structure of below-ground bacterial communities. Despite these results, these authors found that changes in the bacterial community under different lichen-BSCs were not responsible for differences in the ecosystem functionality measured by a suite of different enzyme activities. However, as noted by Castillo-Monroy et al. (2011b), the conventional methods and experimental designs do not lead to an understanding at high-resolution of the functional role of BSCs and their associated microbial community. A great part of the studies dealing with BSCs functionality has been restricted to the measurement of several enzyme activities and functional indicators (i.e. soil respiration) that only provide an overall estimation of soil activity (Miralles et al., 2012a,b). The evaluation of carbon-substrates utilization by community-level physiological profiles (CLPPs) revealed also that biocrusts have a higher level of functional biodiversity than bare soils and that biocrusts may provide soils with a higher capacity to recover from harmful processes by increasing the heterotrophic microbial diversity (Delgado-Baquerizo et al., 2013).

Information about the phylogenetic and functional roles of BSCs and their relationship with the associated soil microbial community is still rather limited. In this study, we evaluated the structure, biomass, and functionality of the microbial community in two BSCs and in the soil underlying these crusts. For this purpose, a multidisciplinary approach was used. Overall, the impacts of BSCs on the ecosystem functionality were addressed by studying the soil respiration and enzyme activities. Furthermore, information regarding the biomass and community structure was obtained by analyzing the PLFAs (Frostegard et al., 1993), and the potential microbial functionality was evaluated on the basis of the substrate utilization by CLPPs profiles. In addition, and since metaproteomics enables extensive characterization of the complete suite of proteins expressed by a community and provides functional information (VerBerkmoes et al., 2009), a metaproteomic study was performed with the aim of obtaining functional information about the BSCs.

We hypothesized that: i) the functionality, biomass, and structure of the microbial community of each biocrust would be different; and ii) the structure and functionality of the microbial communities in the biocrust and soil would differ, and that each BSC would have a distinct impact on the below-ground soil microbial community.

2. Material and methods

2.1. Study area, experimental design, and soil sampling

The study area was located in the province of Murcia (South-East Spain), a region with a Mediterranean semiarid climate and greatly affected by soil degradation processes. The annual average temperature and precipitation are 19 °C and 250 mm, respectively. The vegetation of this area is an open shrub (2–4%) of desertic appearance and biological crusts dominated the inter-shrub vegetation spaces. The soil substrate was a Calcic regosol (Soil Survey Staff, 1998) with pH of 7.98, total organic C content of 0.37 g 100 g^{-1} soil, and total N content of 0.06 g 100 g^{-1} soil.

Two main types of biological crusts, dominated by the lichens *Fulgensia desertorum* (Tomin) Poelt and *Squamarina cartilaginea* (With.) P. James, respectively were identified within a sampling area of 500 m^2. *F. desertorum* is a yellow crustose lichen, while *S. cartilaginea* is a squamulose white lichen.

The upper few centimeters of the surface, corresponding to each biocrust (0–0.5 cm), were extracted. Once the crust was isolated, soil samples (0.5–5 cm) were obtained. Samples were named as follows: *F. desertorum* -dominated biocrust layer (F-BSC); *S. cartilaginea* -dominated biocrust layer (S-BSC); soil below *F. desertorum* (F-soil); and soil below *S. cartilaginea* (S-soil).

Sampling was performed in triplicate in May 2013. Each replicate was composed of 8 sub-samples, to minimize the spatial variability. The samples were sieved to 2 mm. The samples were transported to the laboratory and stored at 4 °C for activity tests and PLFA analysis. Samples for proteomic analyses were stored at −20 °C.

2.2. Chemical, microbiological, and biochemical analysis

The total nitrogen (N) content was determined using Kjeldhal's method as modified by Bremmer and Mulvaney (1978). The total organic carbon (TOC) was determined by oxidation with K_2CrO_7 in an acid medium and titration of the excess dichromate with $(NH_4)_2Fe(SO_4)_2$ (Yeomans and Bremmer, 1989). The pH was measured in a 1/5 (w/v) aqueous extract.

Respiration was analyzed by placing 20 g of sample, at 40–50% of its water holding capacity (water retained at −0.055 MPa), in hermetically-sealed flasks and incubating for 30 days at 28 °C in darkness. The CO_2 released was measured periodically (every day for the first 4 days and then weekly) using an infrared gas analyzer (CheckmateII, PBI Dansensor, Denmark). The cumulative CO_2 evolved during the 30 days of incubation was expressed as mg CO_2–C kg^{-1} soil day^{-1}.

Dehydrogenase activity was determined, in 1 g of sample, as the reduction of 0.2 ml of *p*-iodonitrotetrazolium chloride (INT, 0.4% w/v) to *p*-iodonitrotetrazolium formazan (INTF) at room temperature; the concentration of the INTF formed was estimated by measuring the absorbance in a spectrophotometer at 490 nm.

Urease activity was determined by the buffered method of Kandeler and Gerber (1988). In this procedure, 0.5 ml of a solution of urea (0.48%) and 4 ml of borate buffer (pH 10) were added to 1 g of sample in hermetically-sealed flasks and then incubated for 2 h at 37 °C. The ammonium released was determined by a modified indophenol-blue reaction (Nannipieri et al., 1978) based on the original method published by Fawcett and Scott (1960). Controls were prepared without substrate to determine the amount of ammonium produced in the absence of added urea.

β-glucosidase activity was determined by following the method described by Eivazi and Tabatabai (1987): 2 ml of MUB (pH 6) and 0.5 ml of 0.025 M *p*-nitrophenyl β-D-glucopyranoside were added to 0.5 g of sample. Then, the mixture was incubated at 37 °C for 1 h, and the reaction product, *p*-nitrophenol, was measured at 400 nm.

The specific enzyme activities, defined as the enzyme activity divided by total organic C, and the CO_2 emitted per unit of organic carbon were calculated (Miralles et al., 2012b).

Biolog EcoPlates (Biolog, Inc., Hayward, CA, USA), containing 31 different C sources and water, were used to determine the microbial community-level physiological profiling based on carbon source utilization (Hitzl et al., 1997). One-gram samples were shaken in